Meiotic recombination: **Making and breaking go hand in hand**

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Accurate segregation of homologous chromosomes at the first meiotic division requires the tight coordination of DNA replication, homologous recombination and chromosome organization. Recent studies suggest that the initiation of meiotic recombination is mechanistically coupled to premeiotic DNA replication.

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Current Biology 2001, 11:R45–R48

0960-9822/01/$ – see front matter

Sexually reproducing organisms use the specialized cell division pathway of meiosis to reduce their genome complement by half to generate haploid gametes. During meiosis, a single round of DNA replication is followed by two successive rounds of chromosome segregation. At the first division, homologous chromosomes of maternal and paternal origin — homologs — segregate from one another. Sister chromatids segregate at the second division. Between premeiotic DNA replication and these divisions lies an elongated prophase characterized by a dynamic series of changes in chromosome architecture. In most organisms, a large number of homologous recombination events occur during early prophase, a subset of which become reciprocal exchanges between homologous, non-sister chromatids. These exchanges provide physical links between homologs that allow them to segregate properly at the first division [1].

Proper execution of the meiotic program requires that replication, recombination and the development of higher-order chromosome structures are coordinated with one another. It has been known for some time that meiotic recombination in the budding yeast *Saccharomyces cerevisiae* does not occur if premeiotic DNA synthesis is disrupted, whether by mutation or pharmacological intervention [2–5]. But it was not known whether this reflected a replication-checkpoint-dependent regulatory block to meiotic progression, or a more fundamental mechanistic coupling between replication and recombination. Recent studies [5–9] — one reported elsewhere in this issue of *Current Biology* [6] — shed light on this issue. First, it has been shown that the link between replication disruption and recombination failure is not dependent on checkpoint-mediated cell-cycle arrest [5–8]. Second, there appears to be a strict temporal correlation between DNA replication and recombination initiation on a region-by-region basis [8], and normal progression through premeiotic S phase requires Spo11, a protein directly involved in recombination initiation [9]. These results are consistent with the idea that there is a direct mechanistic link between these processes.

Meiotic recombination is connected to premeiotic DNA replication

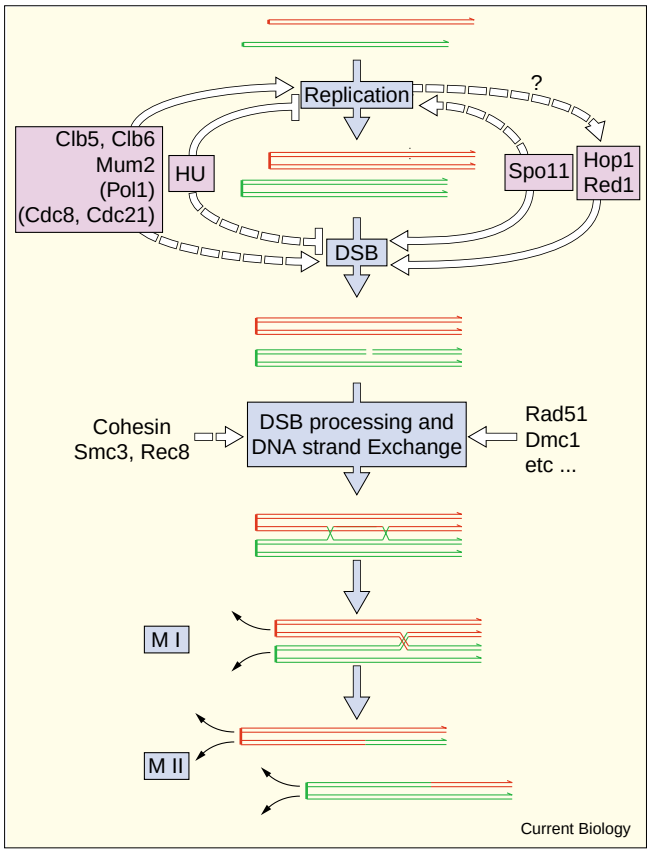
The molecular mechanism of meiotic recombination has been dissected in detail in *S. cerevisiae* [10] (Figure 1). Recombination initiates through the formation of numerous programmed DNA double-strand breaks, which appear to be generated by the topoisomerase-like Spo11 transesterase. These double-strand breaks are repaired by homologous recombination so as to favor interactions between non-sister chromatids.

There is growing evidence that recombination correlates in some way with the ability to carry out premeiotic DNA replication in *S. cerevisiae*. For instance, recombination does not occur in mutants, such as *cdc8*, *cdc21* or *pol1*, that do not replicate their DNA [3,4]. Treating cells with hydroxyurea, which blocks DNA replication by inhibiting ribonucleotide reductase, also prevents meiotic recombination [2,5]. Recent studies indicate that these recombination blocks occur at or prior to the initiation step. Borde and colleagues [8] have shown that hydroxyurea treatment prevents double-strand break formation. Similarly, double-strand break formation does not occur in cells carrying mutations in both *CLB5* and *CLB6* [6].

CLB5 and *CLB6* encode two B-type cyclins, which activate the cyclin-dependent protein kinase Cdc28 to promote the transition from G_1 to S phase in vegetative cells [5]. Premeiotic DNA replication is completely abolished in *clb5/clb5 clb6/clb6* double mutant diploids, although replication is only partially compromised in *clb5/clb5* single mutants and is hardly affected at all in *clb6/clb6* single mutants, indicating that these genes are partially redundant with one another [5,11]. Severe defects in both premeiotic DNA replication and double-strand break formation are also seen in *mum2/mum2* mutants [7,12]. Although the molecular function of Mum2 is not currently understood, it may also play a role in DNA replication in vegetative cells [7].

Taken together, these results demonstrate a correlation between the ability to carry out premeiotic DNA replication and the ability to make double-strand breaks. It is tempting to infer that recombination initiation depends directly upon completion of DNA replication, but the current data do not allow us to draw such a conclusion. For example, it cannot be excluded that the Clb5 and Clb6 cyclins play a more direct role in promoting double-strand break formation that is independent of their role in initiating premeiotic S phase.

Figure 1



Overview of the meiotic recombination pathway in yeast. A pair of homologous chromosomes is cartooned in red and green. After premeiotic DNA replication, a double-strand break (DSB) on one of the chromatids from one homologue is repaired by recombination with a chromatid from the other homologue. Recombination proceeds through a double Holliday junction intermediate which can be resolved as a reciprocal exchange (crossover). At the first meiotic division (MI), the pair of red sisters separates from the pair of green sisters. At the second division (MII), the sister chromatids segregate from one another. Some of the proteins required for this process are indicated: Clb5, Clb6, Mum2, Pol1, Cdc8 and Cdc21 are all required for premeiotic DNA replication. They are also required for double-strand break formation, but it is not certain whether this is because DNA replication itself is required, or because these proteins have other, more direct roles in recombination initiation. Treating cells with hydroxyurea (HU) blocks both DNA replication and double-strand break formation. Spo11 is thought to catalyze double-strand break formation, but it is also required for normal progression through premeiotic S phase. Hop1 and Red1 are meiotic chromosome structure proteins that are required for normal double-strand break formation. Their behavior may be influenced, directly or indirectly, by progression through premeiotic S phase.

The replication–recombination connection is independent of a DNA replication checkpoint

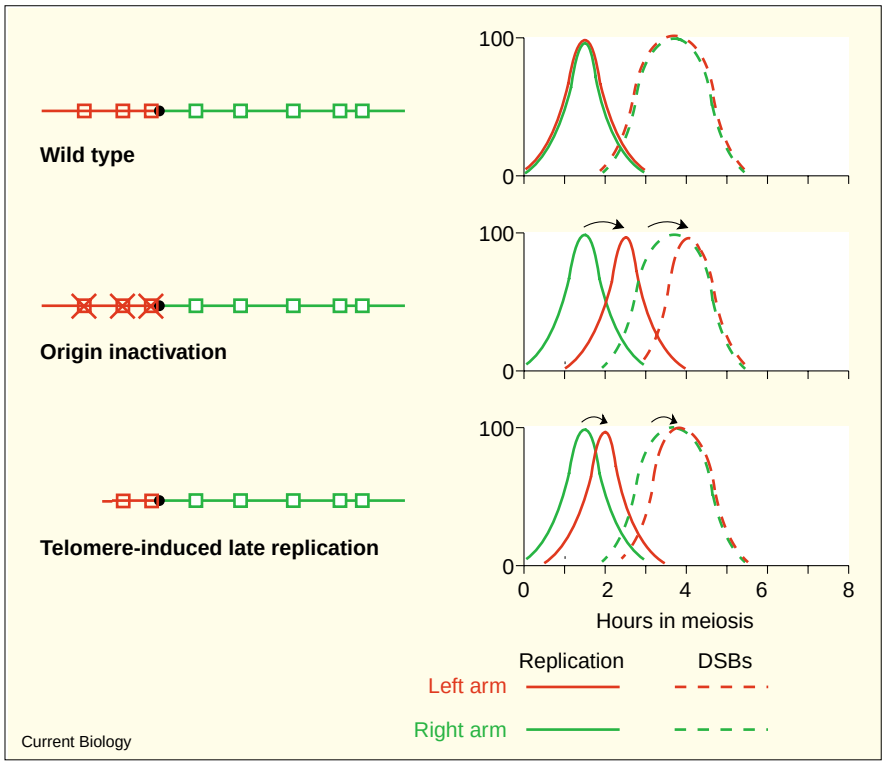
Why is recombination initiation prevented in situations where premeiotic replication does not occur? One possible explanation would be that replication failure invokes a regulatory mechanism — a cell-cycle checkpoint — which in turn prevents recombination by blocking progression through meiotic prophase. Several lines of evidence, however, suggest that this idea is not sufficient to explain the above observations. Hydroxyurea treatment in meiosis blocks cell-cycle progression and prevents cells from dividing, just as it does in vegetative cells [2,8]. In both mitotic and meiotic cells, this arrest requires a functional DNA-replication checkpoint, of which *MEC1* is a key component [5,8]. If the hydroxyurea-induced block to meiotic recombination were an indirect consequence of invoking this checkpoint-mediated arrest, one would expect checkpoint-defective *mec1/mec1* mutants to be proficient for recombination in the presence of hydroxyurea. But they are not [8]. Similarly, the meiotic recombination defects conferred by *mum2* mutations are not alleviated by a *mec1* mutation [7]. Even more striking, failure to replicate DNA in *clb5/clb5 clb6/clb6* double mutants does not trigger the *MEC1*-dependent replication checkpoint at all [5,11], so the recombination defect in these mutants cannot be a consequence of inducing this checkpoint.

Meiosis and sporulation in *S. cerevisiae* involve induction of a specific transcriptional program [13]. Importantly, Smith and colleagues [6] have shown that *clb5/clb5 clb6/clb6* double mutants still express 18 early meiotic genes, including known double-strand-break-promoting elements, such as *SPO11*. Similarly, *mum2/mum2* mutants express the early meiotic gene *HOP1* [7]. These results indicate that the recombination defect in these mutants is not simply a consequence of a failure to induce the meiotic transcriptional program. It should be noted, however, that post-transcriptional expression defects, or a defect in transcription of some other specific gene, cannot be ruled out by these studies. Moreover, the possibility that hydroxyurea treatment induces defects in early meiotic gene expression has not yet been systematically addressed.

Relative timing of replication and double-strand break formation is controlled on a regional, not cell-wide, basis

An elegant series of experiments in *S. cerevisiae* has recently demonstrated a temporal and spatial correlation between premeiotic DNA replication and double-strand break formation, hinting at a more fundamental mechanistic link between the two processes. Borde and colleagues [8] showed that double-strand break formation on yeast chromosome III follows DNA replication after an interval of 1.5–2 hours in normal meiosis. By specifically deleting origins of replication on the left arm of chromosome III, they were able to delay the time of replication of that arm by about one hour, without affecting replication timing in the right arm (Figure 2). Under these circumstances the left arm is presumably duplicated passively by replication forks that have to come in from origins on the right arm. Remarkably, the formation of meiotic double-strand breaks was also delayed on the late-replicating left arm, again by about one hour. The number of double-strand

Figure 2



The time between replication and recombination initiation is constant. On the left are diagrammed a series of configurations of yeast chromosome III: the wild-type configuration; a version with replication origins on the left arm deleted; and a version in which the telomere has been juxtaposed to internal sequences on the left arm. Origins of replication are shown as boxes, the centromere as a black circle. The graphs show the kinetics of appearance and disappearance of premeiotic DNA replication intermediates (solid lines) and double-strand breaks (DSBs, dashed lines) for the left arm (in red) and the right arm (in green) for each chromosome version [8]. Replication of the left arm is delayed if replication origins are inactivated or juxtaposed to the telomere. Double-strand break formation is similarly delayed in these regions, so the interval between replication and double-strand break formation is held constant at roughly 1.5–2 h. In contrast, double-strand breaks disappear (as they are processed into further recombination intermediates) at the same time in all cases. These results suggest that double-strand break formation is controlled on a chromosome regional basis, whereas further steps in recombination are controlled on a cell-wide basis.

breaks was not affected, and the timing of double-strand break formation on the normally replicating right arm was not changed. Similar results were obtained when replication of sequences in the middle of the left arm was delayed by juxtaposing them to a telomere (Figure 2), which is known to cause replication origins to fire later than they normally would [14]. In this case, both replication and double-strand break formation were delayed by about 30 minutes.

These results indicate that the interval between DNA replication and subsequent double-strand break formation is held constant for each chromosomal region, suggesting that the timing of recombination initiation is controlled on a region-by-region basis. In contrast, the processing of double-strand breaks into further recombination intermediates appears to be controlled on a cell-wide basis, rather than regionally, because double-strand breaks disappear (and strand-exchange products appear) at the same time in both late-replicating and normal-replicating chromosome regions (Figure 2).

A mechanistic coupling?

The region-by-region temporal correlation between replication and double-strand break formation, combined with the absence of a clear regulatory (checkpoint) connection, lends support to earlier proposals [9] that there is a mechanistic connection between the two processes. One interpretation of the results is that DNA replication is a rate-limiting step that initiates a series of events that culminate, 1.5–2 hours later, in double-strand break formation [8].

What could the intervening events be? One possibility is that replication is tied to the establishment of higher-order chromosome structures that are required for double-strand break formation. There is ample evidence that cohesion between sister chromatids is established in parallel with replication [15]. Such cohesion structures seem unlikely to provide a direct link between replication and double-strand break formation, however, because double-strand breaks form with normal frequency and kinetics in *smc3/smc3* and *rec8/rec8* mutants, which are defective for components of the cohesin complex [16]. Another prominent chromosome structure that forms during early prophase is the proteinaceous axial element, which forms along the chromosome axis linking a pair of sister chromatids, eventually becoming the lateral element of the synaptonemal complex. Red1 and Hop1 are chromosome structure proteins required for formation of the axial element, and they are also required for forming normal levels of meiotic double-strand breaks [17]. It is unlikely that formation of an axial element *per se* is required for double-strand break formation, because double-strand breaks form normally in many circumstances where axial

element formation is defective (for example, in a *rec8/rec8* mutant [16]). But it may be that early Red1/Hop1-dependent chromosome structures form coordinately with DNA replication and are subsequently required for formation of both axial elements and double-strand breaks.

Another possibility, not exclusive with the first, is that some of the factors directly involved in double-strand break formation are loaded onto the chromosome as the DNA is replicated. Recent studies have established that the duration of premeiotic S phase is shortened in a *spo11Δ/spo11Δ* mutant [9]. Normal progression through premeiotic S phase thus requires the presumed catalytic subunit of the meiotic double-strand break apparatus. This result may indicate that a pre-double-strand break complex — including Spo11 — assembles on chromosomes during DNA replication. Further support for this idea comes from the studies of the cyclin-defective mutants. Most, if not all, double-strand break sites are nuclease-hypersensitive regions in both mitotic and meiotic chromatin [18,19]. Hypersensitivity to micrococcal nuclease increases specifically at double-strand break sites early in meiosis, even before double-strand break formation, perhaps reflecting the assembly of some components of the double-strand break machinery [19,20]. This meiotic increase in nuclease hypersensitivity does not occur in *clb5/clb5 clb6/clb6* cells [6].

These recent studies are providing tantalizing clues about the linkage between premeiotic DNA replication and the initiation of recombination. The roughly two-hour window between replication and double-strand break formation provides ample time for a complicated series of events that remains to be elucidated. It will be exciting to see what details are revealed by further peeks into this window.

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